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## **Ligand Size Distribution of Phenanthroline-functionalized Polyethylene glycol-iron(II) Complexes Determined by Electrospray Ionization Mass Spectrometry and Computer Simulation**

Ákos Kuki, Miklós Nagy, Lajos Nagy, Miklós Zsuga, Sándor Kéki\*

*<sup>1</sup>Department of Applied Chemistry, University of Debrecen, H-4010 Debrecen, Hungary,*

### **Abstract**

The complex formation in solution, and the gas-phase dissociation of a phenanthroline-terminated poly(ethylene glycol) with  $\text{Fe}^{2+}$  ions were investigated. The size distribution of poly(ethylene glycol)- $\alpha$ -monomethyl- $\omega$ -5-[1,10]phenanthroline (mPEG\_phen) was determined by Electrospray Ionization Mass Spectrometry (ESI-MS). Based on the measured ligand size distribution of mPEG-phen by ESI-MS, the 1:3 complex formation ( $\text{Fe}^{2+}/\text{mPEG\_Phen}$ ) was computer-simulated as a pure random assembly process. The simulated distribution fits excellently to that of the complex  $\text{Fe}(\text{mPEG\_Phen})_3^{2+}$  determined from the ESI-MS intensities. In addition, the collision-induced dissociation (CID) of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex was also studied by tandem mass spectrometry (ESI-MS/MS) and by computer simulation, as well. The ESI-MS/MS intensity distribution of the  $\text{Fe}(\text{mPEG\_phen})_2^{2+}$  formed from  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  by the loss of an mPEG\_phen ligand under CID conditions fits quite well to the calculated one.

**Keywords:** PEGphenanthroline-iron(II) complex, collision-induced dissociation, electrospray ionization, mass spectrometry

### **Introduction**

Electrospray Ionization Mass Spectrometry (ESI-MS) [1] offers a unique tool for the investigation of metal-containing complexes because it overcomes the propensity of these complex ions to dissociate when transferred from solution into the gas-phase [2–8]. In addition, the gas-phase complex ions of interest can then be selected and subjected to collision-induced dissociation (CID) to promote the detachment of the intact ligands. In this way, the determination of the relative gas-phase stability of the different complex ions is feasible as it has been shown in several papers [6–9]. Although various types of complexes have been used in several ESI-MS studies, to our knowledge, no ESI-MS report on complexes with ligands consisting of polymer chains has appeared. Since a real polymer is composed of polymer chains with varying chain lengths, it is interesting to study the effect of the chain length on the complex formation and dissociation of these complexes in the gas-phase by utilizing the capability of ESI-MS.

In this paper, we report the ESI-MS and computer simulation studies of the complex formation of a phenanthroline-functionalized polyethylene glycol with  $\text{Fe}^{2+}$  ion and the dissociation of this complex under CID conditions.

## Experimental

### *Materials*

HPLC grade methanol (Scharlau, Sentmenat, Spain), Mohr's salt (Spektrum 3D, Debrecen, Hungary), sodium trifluoroacetate and 5,6-epoxy-5,6-dihydro-[1,10]phenanthroline (Sigma-Aldrich, Steinheim, Germany) were used without further purification. Poly(ethylene glycol) 1100 monomethyl ether was purchased from Fluka (Buchs, Switzerland). The structure of poly(ethylene glycol)- $\alpha$ -monomethyl- $\omega$ -5-[1,10]phenanthroline ether (mPEG\_phen) is presented in Scheme 1. The detailed synthesis of mPEG\_phen will be described in a separate paper.

Scheme 1.

### *Methods*

*Electrospray Quadrupole Time-of-Flight Mass Spectrometry (ESI-QTOF MS).* Measurements were performed with a MicroTOF-Q-type Qq-TOF MS instrument equipped with an ESI source (Bruker Daltonics, Bremen, Germany). The solutions at a concentration of 10  $\mu\text{M}$  were introduced directly into the ESI source with a syringe pump (Cole-Parmer Ins. Comp., Vernon Hills, IL, USA) at a 3  $\mu\text{L}/\text{min}$  flow rate. The  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex was prepared from

Mohr's salt and mPEG\_phen in 1:3 ( $\text{Fe}^{2+}$ /ligand) molar ratio and diluted with methanol to obtain a 10  $\mu\text{M}$  solution. The temperature of the drying gas ( $\text{N}_2$ ) was maintained at 160°C. The needle voltage was kept at 4 kV. For CID experiments  $\text{N}_2$  was used as the collision gas at a pressure of  $8 \times 10^{-3}$  mbar. The precursor ions of interest were selected with a mass window of 6 Daltons and collision voltages of 50-100 V were applied. All the mass spectra were calibrated externally using the exact masses of clusters  $[(\text{NaTFA})_n + \text{Na}]^+$  generated from the electrosprayed solution of sodium trifluoroacetate (NaTFA). The mass spectra recorded were evaluated by the DataAnalysis 3.1 software from Bruker.

*Computer simulation.* The simulation of the complex formation is given in the Supplemental Information (Algorithm 1). The ligands array contains the ligand length values, each element represents a ligand. Three ligands are chosen from this array randomly to form a complex. The consumed ligands are deleted from the ligands array. After assembling a complex, the appropriate element of the complexes counter array is incremented.

The computer simulation procedure of the fragmentation process of the PEG-phenanthroline-iron(II) complex ions is presented in the Supplemental Information (Algorithm 2). The complexes with 84 ethylene oxide repeat units are chosen and their lengths are decreased with the length of a randomly selected ligand. Then, with the appropriate element of the fragmented complexes a counter array is incremented. Two consecutive ligand losses can be simulated by a slight modification of Algorithm 2.

## Results and Discussion

Our preliminary goal was to study the relationship between the ESI-MS intensity of poly(ethylene glycol)- $\alpha$ -monomethyl- $\omega$ -5-[1,10]phenanthroline ether (mPEG\_phen) and that of the  $\text{Fe}(\text{mPEG-phen})_3^{2+}$  complex. Fig. 1 shows the normalized distribution of the ligand mPEG\_phen as a function of the number of repeat units (ethylene oxide) as determined from the corresponding ESI-MS intensities.

Fig. 1.

As seen in Fig. 1, mPEG\_phen is composed of polymer chains with varying number of repeat units ( $n$ ) that span from  $n=19$  to  $n=38$  and centered at  $n=28$ . In the next experiment a tris-complex of  $\text{Fe}^{2+}$  was prepared using the mPEG\_phen ligand whose distribution is presented in Fig 1. The ESI-MS spectrum of the tris-complex of  $\text{Fe}^{2+}$  is shown in Fig. 2.

Fig. 2.

As Fig. 2 shows, the tris-complex occurs with different charge states in the ESI-MS spectrum. For example, doubly charged ions originate from the complex  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$ , while the triply and quadruply charged ones are due to the additional attachment of one and two  $\text{NH}_4^+$  ions, respectively, to the PEG chains of the tris-complex. The presence of  $\text{NH}_4^+$  ions are due to the Mohr's salt used for the preparation of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex, as it was shown that  $\text{NH}_4^+$  is able to attach to the polyether chains [10]. In the following, we determined the intensity distribution for the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complexes from the ESI-MS spectrum as a function of the total number of the repeat units. To obtain the intensity distribution of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complexes, the ESI-MS intensities at different charged states were summed and then compared to the distribution calculated on the basis of the procedure to be presented below. It is to be noted, however, that consecutive additions of the intensities of charge states from +3 to +6 to those of charge state +2 does not significantly affect the resulting intensity distribution (see the Supplemental Information).

The formation of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complexes can be given by eq. 1,



where L is the ligand, mPEG\_phen and the subscripts represent the numbers of the repeat units.

To calculate the expected intensity distribution for  $\text{Fe}(\text{mPEG-phen})_3^{2+}$  as a function of the total number of repeat units, i.e.,  $x+y+z$  according to eq. 1, we applied a short computer program presented in the Supplemental Information. Our program worked with mPEG\_phen particle quantity of 660000 distributed according to that shown in Fig. 1.

Three mPEG\_phen particles were chosen randomly to form a  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex ion. All of the mPEG\_phen was consumed during the simulation process, so 220000  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex ions were formed.

Fig. 2. inset shows the measured and the calculated intensity distributions for the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complexes. As seen in Fig. 2 inset, the calculated intensity distribution fits excellently to the experimental one. Alternatively, we also determined the distribution for the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complexes by calculating the probabilities of formation of tris-complexes with a particular chain length according to eq. 2

$$P_n = \sum_{i,j,k=19}^{38} P_i P_j P_k \quad (2)$$

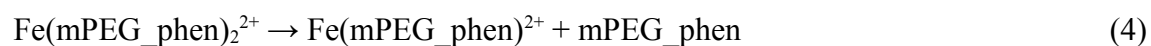
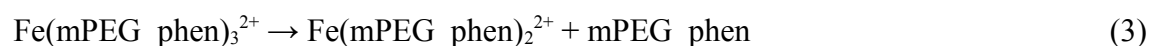
where  $P_n$  is the probability of the formation of a complex which carries  $n$  ethylene oxide repeat units altogether in the three ligands.  $P_i$ ,  $P_j$  and  $P_k$  are the probabilities in the chain length distribution of mPEG\_phen – presented in Fig. 1 – with  $i$ ,  $j$  and  $k$  repeat units and  $i+j+k=n$ .

The intensity distributions for the  $\text{Fe(mPEG\_phen)}_3^{2+}$  complexes calculated by the two methods yield the same results. Based on the excellent coincidence of the measured and calculated distributions, it can be concluded that the formation of the  $\text{Fe(mPEG\_phen)}_3^{2+}$  complex is independent of the mPEG\_phen chain length, i.e., there is no significant difference in the binding energy of mPEG\_phen of different sizes.

In further studies, the  $\text{Fe(mPEG\_phen)}_3^{2+}$  complex was subjected to collision-induced dissociation (CID). The product ion spectrum (MS/MS) of the  $\text{Fe(mPEG\_phen)}_3^{2+}$  ion with a total number of repeat units 84 is shown in Fig. 3.

Fig. 3.

As it can be seen in Fig. 3, two series of peaks denoted by A and B appeared in the MS/MS spectrum. The  $m/z$  difference between the adjacent peaks in each series is  $m/z$  22; corresponding to the mass to charge ratio of an ethylene oxide unit bearing two charges. On the other hand, accurate mass measurements support that no cleavage of the backbone of the ligand takes place. Hence, the product ion series A and B are formed by the loss of one and two intact ligands from the precursor ion in a consecutive process under CID as depicted below:



The product ions of series A reveal an intensity distribution centered at  $m/z$  1493 corresponding to the bis-complex  $\text{Fe(mPEG\_phen)}_2^{2+}$  with a total number of ethylene oxide units 57. The occurrence of this series can be attributed to the process described by eq. 3, while the low intensity series B is due to the consecutive dissociation of  $\text{Fe(mPEG\_phen)}_2^{2+}$

(eq. 4). It is important to clarify that in spite of the fact that only one particular precursor complex ion was selected, the resulting product ion spectrum exhibits many peaks which may be associated with the distribution of mPEG\_phen in the complex ion. For example, a tris-complex with 84 ethylene units altogether can be formed from mPEG\_phen chains having 23, 28, and 33 units and/or bearing 27, 32, and 25 units, and so forth, generating lots of combinations. Thus, when a complex with a particular total number of repeat units is subjected to MS/MS the resulting product ion distribution may be related to the mPEG\_phen distribution.

In order to examine whether the loss of the ligand(s) is independent of the mPEG\_phen chain-length, i.e., each fragmentation channel is completely equal, and how the distribution of series A and B are related to that of mPEG\_phen (Fig. 1), our computer simulation was extended to the dissociation of  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$ . As seen in the inset of Fig. 3 the simulated intensity distributions for both series fits quite well to the experimental ones indicating that the dissociation of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex ion does not depend on the size of the ligand and the resulting intensity distributions of the product ions are related to that of mPEG\_phen. It can be also seen from Fig 3. inset that the collision voltage/energy does not affect the intensity distribution of the  $\text{Fe}(\text{mPEG\_Phen})_2^{2+}$  ions. The intensity distribution of  $\text{Fe}(\text{mPEG\_Phen})_2^{2+}$  recorded at 80 V collision voltage (near the dissociation threshold) matches that recorded at 90 V collision voltage. Therefore, the „kinetic shift” does not play a significant role in the dissociation of  $\text{Fe}(\text{mPEG\_Phen})_2^{2+}$  into  $\text{Fe}(\text{mPEG\_Phen})^{2+}$  under our experimental conditions.

## Conclusions

ESI-MS was proved to be capable of the investigation of the complex formation of  $\text{Fe}^{2+}$  with the polymer ligand mPEG\_phen, and ESI-MS/MS can be used for studying the dissociation properties of these complexes in the gas-phase. Based on our experiments and model calculations, it is concluded that both the formation and the collision-induced dissociation of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex can be described in a simple way. Furthermore, such kind of ESI-MS and ESI-MS/MS investigations can be extended to another complexes containing metal and polymer ligands, as well.

## Acknowledgment

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## Legends for the Scheme and Figures

### Scheme 1.

The structure of poly(ethylene glycol)- $\alpha$ -monomethyl- $\omega$ -5-[1,10]phenanthroline ether (mPEG\_phen)

### Fig. 1.

The normalized intensity distribution of the mPEG\_phen as a function of the number of repeat units. The normalized intensities ( $I_{\text{norm},i}$ ) were obtained as:  $I_{\text{norm},i} = I_i / \sum I_i$  where  $I_i$  is the ESI-MS intensity of mPEG\_phen with a number of repeat units  $i$  and  $\sum I_i$  is the sum of all mPEG\_phen intensities. (The ESI-MS signals are originated from the sodiated oligomers). Note that this normalized intensity distribution expresses also the probability of finding mPEG\_phen chain with a number of repeat unit  $i$ .

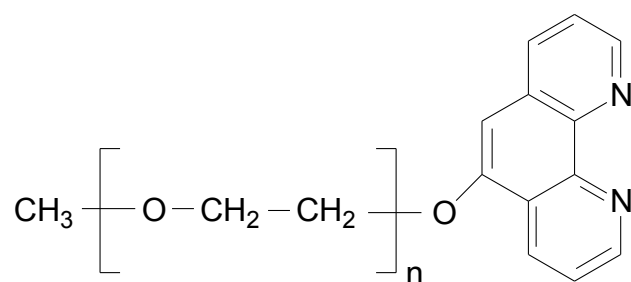
### Fig. 2.

ESI-MS spectrum of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex. The inset shows the measured and the calculated normalized intensity distributions for the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  as a function of the total number of repeat units. L stands for the mPEG\_phen ligand. The normalized intensities ( $I_{\text{norm},i}$ ) were obtained as:  $I_{\text{norm},i} = \sum_j I_{i,j} / I_{\text{max}}$  where  $\sum_j I_{i,j}$  is the sum of intensities of  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  containing a total number of repeat units  $i$  in charge states ( $j$ ) ranging from +2 to +6.  $I_{\text{max}}$  is the maximum intensity obtained after summation.

### Fig. 3.

Product ion spectrum (MS/MS) of the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$  complex with a number of ethylene oxide units 84 ( $m/z$  2193). The numbers in the subscripts represent the number of the ethylene oxide units. The inset shows the measured intensity distributions obtained at collision voltages 80 V and 90 V together with the calculated intensity distribution of the product ions formed from the  $\text{Fe}(\text{mPEG\_phen})_3^{2+}$ .

**Scheme 1.**



**Fig. 1.**

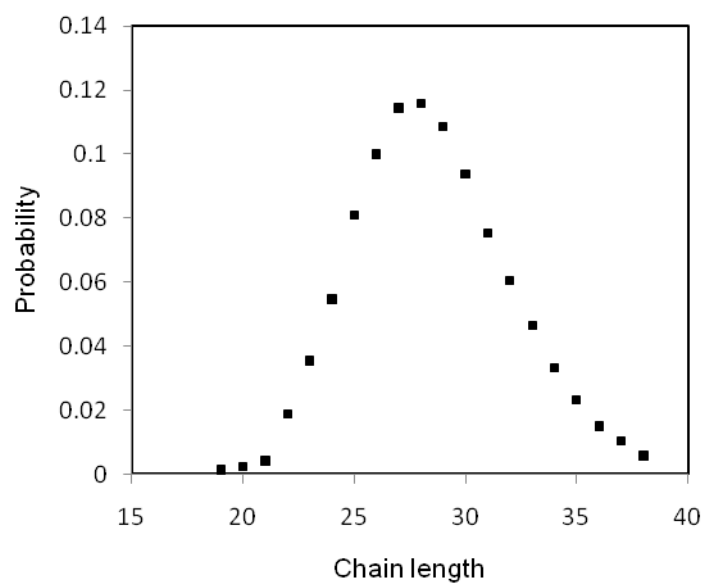


Fig. 2.

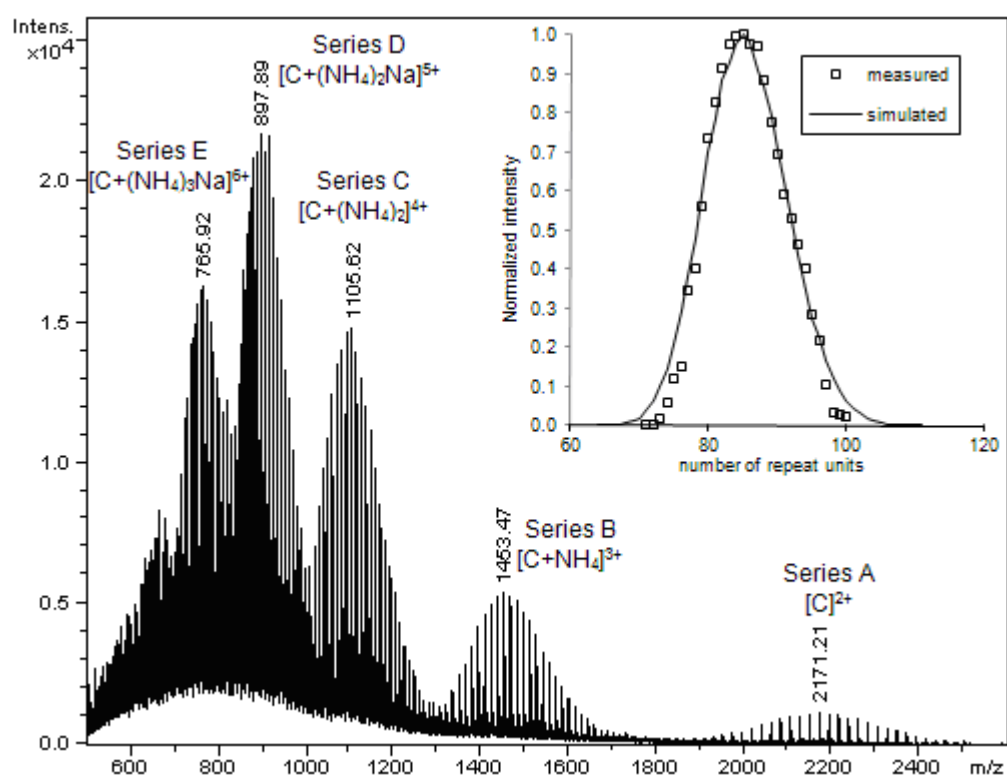


Fig. 3.

